

# NEW REFERENCE MATERIALS FOR NITROGEN-ISOTOPE-RATIO MEASUREMENTS

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Three new reference materials were manufactured for calibration of relative stable nitrogen-isotope-ratio measurements :

USGS25 (ammonium sulfate)  $\delta^{15}\text{N}' = -30$  per mil

USGS26 (ammonium sulfate)  $\delta^{15}\text{N}' = +54$  per mil

USGS32 (potassium nitrate)  $\delta^{15}\text{N}' = +180$  per mil

where  $\delta^{15}\text{N}'$ , relative to atmospheric nitrogen, is an approximate value subject to change following interlaboratory comparisons. These materials are isotopically homogeneous in aliquots at least as small as 10  $\mu\text{mol N}_2$  (or about 1-2 mg of salt). The new reference materials greatly extend the range of  $\delta^{15}\text{N}$  values of internationally distributed standards, and they allow normalization of  $\delta^{15}\text{N}$  measurements over almost the full range of known natural isotope variation on Earth. The methods used to produce these materials may be adapted to produce homogeneous local laboratory standards for routine use.

The primary standard for relative nitrogen-isotope-ratio measurements is atmospheric nitrogen gas, which appears to be homogeneous with respect to current analytical precision in most laboratories (1). A limited number of secondary reference materials (salts, reagent gases, etc.) also have been widely distributed internationally (2). Secondary reference materials with a large range of isotope compositions are essential for reporting or expressing isotope-ratio measurements on normalized scales. They also are useful in many applications when it is desirable to avoid problems associated with purifying nitrogen from air, or when it is important to test laboratory preparation and analysis procedures for solid or liquid samples.

The major problem to be addressed in this paper is that the range of isotope compositions in previously available reference materials is much smaller than the range ob-

served in natural environments on Earth (Figure 1; (3)), so that analyses of many such substances cannot be properly expressed on a normalized scale. This problem is increasingly common in studies of nitrate contamination in shallow ground waters, which account for many of the highest reported  $\delta^{15}\text{N}$  values in Figure 1. We have manufactured three new solid reference materials in large quantities and calibrated them provisionally with respect to atmospheric nitrogen and the international reference materials IAEA-N1, IAEA-N2, and NSVEC. The new materials (USGS25, USGS26, and USGS32) are ammonium and nitrate salts, and they have a wide range of  $\delta^{15}\text{N}$  values that encompasses all but a few of the many thousands of values reported in the literature for terrestrial substances (see Figure 1). Here we describe the preparation procedures and report the results of relative-isotope-ratio measurements and homogeneity tests for the new materials. Provisional  $\delta^{15}\text{N}$  values for materials other than USGS32 are given in NIST (6); recommended values for a full suite of nitrogen-isotope secondary reference materials will be compiled later after interlaboratory analytical comparisons are completed.

## PREPARATION METHODS

### Synthesis of reference materials

The general procedure used to prepare each of the new reference materials was as follows : approximately 1500 g of reagent salt (ammonium sulfate or potassium nitrate) was added to 4 L of de-ionized water (DIW) in a clean smooth polypropylene tub (900  $\text{cm}^2 \times 12$  cm high). The use of large-volume plastic containers with relatively high walls was found to minimize the loss of salts by crystallization up and over the top of the walls that caused problems in experiments with relatively full glass beakers. The

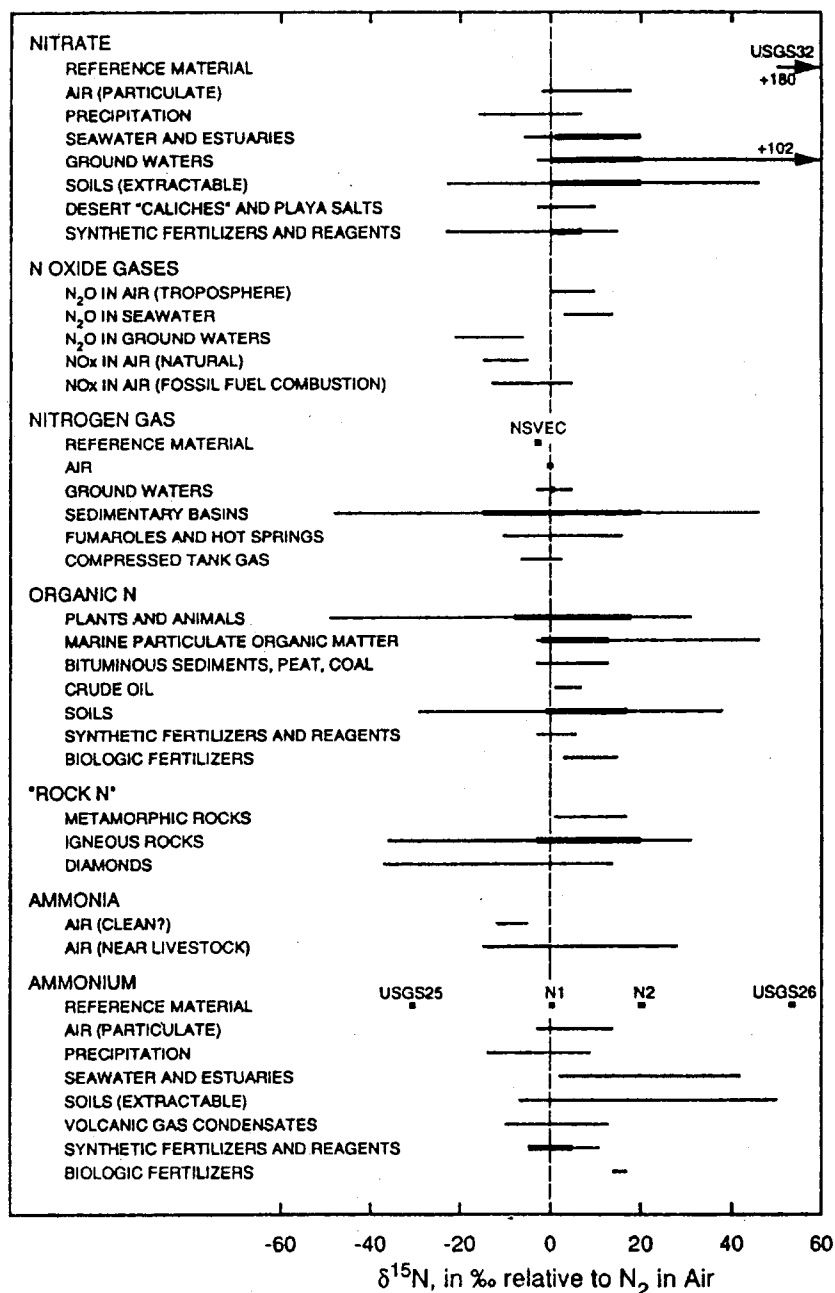


Figure 1. Partial compilation of  $\delta^{15}\text{N}$  values for some categories of substances on Earth (sources of data are given in (3)).  $\delta^{15}\text{N}_{\text{air}}$  is defined as  $[R_{\text{sample}}/R_{\text{air}} - 1] \times 1000$ , where  $R$  is the molar ratio of  $^{15}\text{N}/^{14}\text{N}$  and  $R_{\text{air}} = 1/272.0$  (4, 5). Thin lines indicate ranges of reported values; thick bars indicate typical values for some common substances. Major reference materials distributed before 1992 have  $\delta^{15}\text{N}$  values between approximately -3 and +20 per mil, whereas the range of values reported in the literature from natural environments is at least -49 to +102 per mil. The new USGS reference materials (-30 to +180) allow normalization over almost the full range of reported values

plastic tub containing the salt slurry was placed in a clean shallow glass baking dish on the shelf of a large drying oven with internal fans and an opening at the top and set to  $90 \pm 2^\circ\text{C}$ . After several hours, the plastic tub containing the warm reagent solution was removed from the oven, stirred, and diluted with DIW until the salt dissolved completely. A second solution was prepared by dissolving completely a weighed amount of isotopically enriched or depleted salt [Cambridge Isotope Laboratories (CIL) 99.99%  $^{14}\text{N}$  ammonium sulfate, 10%  $^{15}\text{N}$  ammonium sulfate, or 5%  $^{15}\text{N}$  potassium nitrate] in a beaker containing about 400 mL of DIW. The second solution was added to the reagent solution in the tub, and the mixture was stirred, checked for complete dissolution, and returned to the oven. After water was no longer visible (2-4 days), the dried salt mass in the tub was broken into smaller chunks and returned to the oven. After one more day, the dried salt chunks were ground gently in an alumina mortar and stored temporarily in clean glass bottles with teflon-lined screw caps. Later, the salt was ground gently in an alumina mortar and sieved in stages until it passed completely through a 40-mesh (425  $\mu\text{m}$ ) stainless-steel screen. The ground and sieved salt was then passed through gravity-fed stainless-steel and aluminum splitters to produce homogeneous fractions for distribution. The final splits contain 600-1000 mg of salt and are stored in 13 mL glass bottles with Teflon-lined screw caps.

The salts used to make USGS25 were 1456.5 g of reagent  $(\text{NH}_4)_2\text{SO}_4$  (Fisher A938-500, lot #915021) plus 46.04 g of isotopically enriched  $(\text{NH}_4)_2\text{SO}_4$  (CIL, 99.99%  $^{14}\text{N}$ , lot BI-1328). The salts used to make USGS26 were 1515.0 g of reagent  $(\text{NH}_4)_2\text{SO}_4$  (Fisher A938-500, lot #915021) plus 3.08 g of isotopically enriched  $(\text{NH}_4)_2\text{SO}_4$  (CIL, 10%  $^{15}\text{N}$ , lot BI-1050). The salts used to make USGS32 were 514.8 g of reagent  $\text{KNO}_3$  (Baker Analyzed 3190-01, lot D40117) plus 970.9 g of reagent  $\text{KNO}_3$  (Baker Analyzed 3190-05, lot C19162) plus 20.00 g of isotopically enriched  $\text{KNO}_3$  (CIL, 5%  $^{15}\text{N}$ , lot F-2685).

### Isotope analysis

For preliminary isotope-ratio calibrations and homogeneity tests with relatively large sample sizes, weighed quantities of salts were sealed with a torch into evacuated 9 mm x 15 cm VYCOR glass tubes with 3 g of Cu granules (Alpha Resources AR 263), 2 g of CuO wire (Baker Analyzed 1820), and 0.2 g CaO powder (Fisher C117) (procedures modified slightly from (7)). The contents of the sealed tubes were shaken and mixed with a vibrating tool and the tubes were placed in a muffle furnace that was then heated to  $850^\circ\text{C}$ , held at  $850^\circ\text{C}$  for 2 h, and cooled at

$40^\circ\text{C/h}$  back to room temperature.  $\text{N}_2$  gas that was produced and purified by high temperature reactions in each tube was expanded into the sample inlet of a Finnigan MAT 251 isotope-ratio mass spectrometer and analyzed against a nitrogen reference gas (NSVEC) that has a reported  $\delta^{15}\text{N}_{\text{air}}$  value of -2.8 per mil (7).

Similar procedures were followed for preparation of air samples. Tubes containing combustion reagents (Cu, CuO, and CaO) were evacuated for 30-60 minutes, then the vacuum line was isolated from the vacuum pumps. Laboratory air samples were expanded from a 1-atmosphere reservoir into the vacuum line with the tubes attached, and the total pressure was adjusted with a mercury piston. The tubes were then isolated from the large reservoir and sealed with the torch, mixed, combusted and analyzed along with the salts. No adjustment was made for the effect of atmospheric argon on the nitrogen-isotope measurements (2, 8).

For homogeneity tests of the new reference materials at relatively small sample sizes, the procedures were similar except that the combustion reagents listed above for each sample were replaced with 0.1 g of CaO plus 2 g of a locally produced mixture of Cu and  $\text{Cu}_2\text{O}$ . The new Cu- $\text{Cu}_2\text{O}$  combustion reagent was prepared by baking Cu granules (Alpha Resources, AR 263) in a shallow ceramic dish in air at  $600^\circ\text{C}$  for approximately 3 days, intermittently breaking up and stirring the intergrown mass (procedure modified from that of M. Fogel, Carnegie Institution, oral commun., 1992). This reagent consists of about 60% (by volume) relict Cu in the cores of the granules and about 40%  $\text{Cu}_2\text{O}$  (identified by X-ray diffraction) as replacement rims on the granules with a trace amount of CuO. The altered granules were crushed gently to expose the Cu before being added to the salts and CaO. Thermodynamic calculations and results of homogeneity tests indicate that the Cu- $\text{Cu}_2\text{O}$  mixed reagent is effective at converting both nitrate and ammonium to  $\text{N}_2$  gas.

The locally produced Cu- $\text{Cu}_2\text{O}$  mixture added significantly less nitrogen contamination during combustion than did comparable amounts of Cu plus CuO wire. Reagent blanks containing 1 g of Cu granules plus 1 g of CuO wire (with 0.1 g CaO) yielded approximately 0.06-0.08  $\mu\text{mol}$  of  $\text{N}_2$  with  $\text{N}_2/\text{Ar}$  about 3-5 times that of air and  $\delta^{15}\text{N} = +18 \pm 3$  per mil. Reagent blanks containing 2 g of the locally produced Cu- $\text{Cu}_2\text{O}$  mixed reagent (with 0.1 g CaO) yielded approximately 0.01-0.02  $\mu\text{mol}$  of  $\text{N}_2$  with a  $\text{N}_2/\text{Ar}$  ratio 1 to 2 times that of air and  $\delta^{15}\text{N} = 0 \pm 3$  per mil ( $\delta^{15}\text{N}$  was measured on larger blanks containing 8 g of Cu- $\text{Cu}_2\text{O}$  with 0.4 g CaO).

Table 1. Average  $\delta^{15}\text{N}_{\text{N1}}$  values and standard deviations for some nitrogen-isotope reference materials

Sample Size ( $\mu\text{mol N}_2$ )	Reagents <sup>b</sup>	Air	IAEA-N1	IAEA-N2	USGS25	USGS26	USGS32
$\delta^{15}\text{N}_{\text{N1}}^{\text{a}}$							
		-0.35	+0.00	+19.95	-30.53	+53.24	+179.52
Standard deviation ( $\sigma$ ) in per mil <sup>c</sup>							
120	Cu+CuO	—	—	—	0.01 (n=3)	0.00 (n=2)	0.05 (n=3)
60	Cu+CuO	0.08 (n=6)	0.05 (n=6)	0.11 (n=6)	0.04 (n=6)	0.07 (n=5)	0.17 (n=6)
10	Cu-Cu <sub>2</sub> O	—	—	—	0.04 (n=15)	0.09 (n=15)	0.16 (n=15)

<sup>a</sup> - Average  $\delta^{15}\text{N}$  values in per mil deviation from IAEA-N1; the value given for air was not adjusted for the effects of Ar in the mass spectrometer (2, 8).

<sup>b</sup> - Combustion reagents used for sample preparation: Cu+CuO refers to 2 g of Cu granules plus 2 g of CuO wire plus 0.2 g of CaO powder; Cu-Cu<sub>2</sub>O refers to 2 g of locally-produced Cu-Cu<sub>2</sub>O plus 0.1 g of CaO powder.

<sup>c</sup> - Standard deviations from the mean value of  $\delta^{15}\text{N}$  in per mil deviation from a single aliquot of reference gas; n is the number of randomly selected sample splits that were prepared and analyzed as a group of unknowns; small standard deviations indicate homogeneity of samples and blanks.

## CALIBRATION OF NITROGEN-ISOTOPE RATIOS

For provisional calibration of  $\delta^{15}\text{N}$  values, the new reference materials were prepared in sample sizes corresponding to 60 and 120  $\mu\text{mol}$  of  $\text{N}_2$  and analyzed together with atmospheric  $\text{N}_2$  and with IAEA standards N1 and N2, both of which are ammonium sulfate salts. Those data (Table 1) are reported as mean  $\delta^{15}\text{N}$  values relative to IAEA-N1 (rather than air) to emphasize that the preparation methods and yields of all analyzed salts were identical. Comparisons with atmospheric  $\text{N}_2$  (the primary nitrogen-isotope-ratio standard) may be complicated by mass-spectrometer-specific measurement effects of argon in air, which can cause errors in  $\delta^{15}\text{N}$  measurements of  $\pm 0.1$ -0.2 per mil (2, 8). Therefore, it is likely that comparisons among secondary salt standards (e.g., Table 1) generally will yield simpler and more precise interlaboratory calibrations than will measurements against atmospheric nitrogen.

Some reported values of  $\delta^{15}\text{N}_{\text{air}}$  for secondary nitrogen-isotope reference materials are summarized in Table 2.

The new salts may be used as reference materials for normalizing nitrogen-isotope-ratio measurements from approximately -30 to +180 per mil, which greatly extends the range of standards distributed internationally before 1992 (-3 to +20 per mil). However, internationally accepted (certified or recommended)  $\delta^{15}\text{N}$  values have not been adopted for any of the solid reference materials, despite that fact that some of them have been widely used. Interlaboratory comparisons involving a full suite of secondary nitrogen-isotope reference materials are in progress.

## EVALUATION OF ISOTOPIC HOMOGENEITY

The results of the calibration tests (Table 1) indicate that the new reference materials are isotopically homogeneous in sample sizes corresponding to 120 and 60  $\mu\text{mol N}_2$ . For more rigorous homogeneity tests, 15 splits of each salt were prepared and analyzed with the low-blank Cu-Cu<sub>2</sub>O reagent in quantities corresponding to 10  $\mu\text{mol N}_2$  (1.3 mg ammonium sulfate or 2.0 mg potassium nitrate). Each of the analyzed splits was taken from a different bottle after all of the materials were split for

Table 2. Partial compilation of  $\delta^{15}\text{N}_{\text{air}}$  values for some international nitrogen-isotope reference materials

Identification <sup>a</sup>	Material	$\delta^{15}\text{N}_{\text{air}}$ <sup>b</sup>	Reference
USGS25 (8550)	( $\text{NH}_4$ ) <sub>2</sub> SO <sub>4</sub>	-30.4	Ref.6
		-30.2	this study
IAEA-N1 (8547)	( $\text{NH}_4$ ) <sub>2</sub> SO <sub>4</sub>	+0.4	Ref.6
		+0.44 $\pm$ 0.39 (n=8)	Ref.9
		+0.45	Ref.7
		+0.35	this study
IAEA-N2 (8548)	( $\text{NH}_4$ ) <sub>2</sub> SO <sub>4</sub>	+20.3	Ref.6
		+20.18 $\pm$ 0.70 (n=8)	Ref.9
		+20.35	Ref.7
		+20.30	this study
USGS26 (8551)	( $\text{NH}_4$ ) <sub>2</sub> SO <sub>4</sub>	+53.5	Ref.6
		+53.6	this study
IAEA-N3 (8549)	KNO <sub>3</sub>	+3 $\pm$ 1	Ref.6
USGS32 (8558)	KNO <sub>3</sub>	+179.9	this study
NSVEC (8552)	N <sub>2</sub>	-2.81	Ref.7
NBS14	N <sub>2</sub>	-1.18	Ref.7

<sup>a</sup> - numbers in parentheses are from NIST (Ref.6).

<sup>b</sup> - average or reported  $\delta^{15}\text{N}$  values in per mil deviation from atmospheric N<sub>2</sub>.

distribution. All 15 splits of each material were analyzed against the same aliquot of reference gas (NSVEC). Those data indicate that USGS25 is isotopically homogeneous to within the normal analytical precision of the mass spectrometer ( $\pm 0.02$ - $0.04$  per mil). The analytical uncertainties are slightly larger for USGS26 ( $\pm 0.09$ ) and USGS32 ( $\pm 0.16$ ); however, the magnitudes of the uncertainties are such that they are probably attributable to slight variations in either reagent blanks or air contamination (including possible leakage while admitting the gas to the mass spectrometer), which affect most severely analyses of samples with  $\delta^{15}\text{N}$  values far from that of air. For example, variation in air contamination or the Cu-Cu<sub>2</sub>O blank of  $0.01 \mu\text{mol}$  of N<sub>2</sub> with  $\delta^{15}\text{N} = 0$  per mil could cause variation in the measured  $\delta^{15}\text{N}$  values of the salts by  $0.03$  (USGS25),  $0.05$  (USGS26) or  $0.18$  (USGS32) per mil. Because the standard deviations of the homogeneity tests increase systematically with the difference between  $\delta^{15}\text{N}_{\text{salt}}$  and  $\delta^{15}\text{N}_{\text{air}}$  and are of the same magnitude as the calculated effects of observed variations in the blanks, it is concluded that all three new reference materials are isotopically homogeneous to within analytical precision. Homogeneity tests for smaller sample sizes will be conducted as part of the ongoing interlaboratory comparison.

## DISTRIBUTION INFORMATION

Bottles containing splits of the three new USGS reference materials have been transferred to the U.S. National Institute of Standards and Technology (NIST), Standard Reference Materials Program, Room 204, Building 202, Gaithersburg, Maryland, 20899, U.S.A. for distribution. Splits of the new materials plus IAEA reference materials N1, N2, N3, and NSVEC (Table 2) have been distributed internationally for interlaboratory analytical comparison. NIST catalog numbers for all seven of those reference materials are listed in Table 2.

## CONCLUSIONS

(1) Three new reference materials for nitrogen-isotope-ratio measurements have been manufactured, homogenized, and split for distribution. The new materials greatly extend the range of  $\delta^{15}\text{N}$  values in previously distributed nitrogen-isotope reference materials.

(2) The new materials appear to be isotopically homogeneous for sample sizes at least as small as  $10 \mu\text{mol}$  N<sub>2</sub>.

(3) Provisional  $\delta^{15}\text{N}_{\text{air}}$  values for the new reference materials have been determined, but internationally accepted values for normalization will not be available until

after interlaboratory comparisons are completed. In the meantime, it is suggested that reported  $\delta^{15}\text{N}$  values for natural samples should be accompanied by measured values for reference materials with  $\delta^{15}\text{N}$  values that bracket those of the samples being analyzed.

(4) The solution/precipitation methods used to prepare the new reference materials may be adapted to produce isotopically homogeneous local laboratory standards for routine use.

(5) A new Cu-Cu<sub>2</sub>O combustion reagent for preparation of N<sub>2</sub> gas for nitrogen-isotope analysis was shown to yield significantly smaller nitrogen blanks than did previously suggested sealed-tube combustion reagents.

### ACKNOWLEDGEMENT

We thank F. Brookman, G. Miller and C. Zacko for assistance grinding, splitting, and bottling the salts, and we thank J. Fassett, M. Fogel, J. Kane, K. Revesz, and E. Spiker for constructive comments on the manuscript. Use of firm, brand, and trade names in this paper is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

### RESUME

**Trois nouveaux matériaux de référence ont été fabriqués pour le calibrage de mesures de rapports isotopiques relatifs de l'azote :**

USGS25 (sulfate d'ammonium)  $\delta^{15}\text{N}' = -30$  per mil  
USGS26 (sulfate d'ammonium)  $\delta^{15}\text{N}' = +54$  per mil  
USGS32 (nitrate de potassium)  $\delta^{15}\text{N}' = +180$  per mil  
où  $\delta^{15}\text{N}'$ , relatif à l'azote atmosphérique, est une valeur approximative sujette à changements après des études interlaboratoires. Ces matériaux sont isotopiquement homogènes dans un aliquot aussi petit que 10  $\mu\text{mol N}_2$  (ou environ 1-2 mg de sel). Les nouveaux matériaux de référence étendent considérablement la gamme des valeurs de  $\delta^{15}\text{N}$  des étalons internationaux distribués et ils permettent la normalisation des mesures de  $\delta^{15}\text{N}$  sur presque toute la gamme de variations isotopiques connues sur terre. Les méthodes employées pour produire ces matériaux peuvent être adaptées pour produire des étalons homogènes à usage interne de laboratoire en analyse de routine.

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